WHAT IS NEW AND DESIRED TO BE SECURED BY LETTERS PATENT OF THE UNITED STATES IS:

1. A method for isolating an endotoxin binding protein from a horseshoe crab, which comprises:

subjecting amebocytes obtained from a horseshoe crab to hypotonic shock to lyse said amebocytes, and obtain cell debris from said lysed amebocytes,

extracting said cell debris with a solution containing a denaturant selected from the group consisting of urea and guanidine hydrochloride, to produce an extract,

passing said extract through a first ultrafiltration membrane having a molecular cutoff of from 20,000 to 50,000 daltons, to obtain a filtrate,

concentrating said filtrate by passing it through a second ultrafiltration membrane having a molecular cutoff of from 5,000 to 10,000, to produce a retentate,

subjecting said retentate to cation exchange chromatography at a pH of from about 5 to 6, using an elution buffer which comprises urea, and eluting a solution containing a peak of endotoxin binding activity,

applying said solution containing said peak of endotoxin binding activity to a reverse phase column, and adding a buffer to said reverse phase column, to obtain a solution containing purified endotoxin binding protein.

2. The method according to Claim 1, wherein said hypotonic shock is accomplished by treating said amebocytes with endotoxin-free distilled water at about 0°C to 10°C.

3. The method according to Claim 1, wherein said extraction of said cell debris is accomplished with 6 molar urea or guanidine hydrochloride.

4. The method according to Claim 1, wherein said ultrafiltration membranes are each composed of polysulfone.

5. The method according to Claim 1, wherein before passing said extract through said membrane, the extract is crudely filtered with a filter aid selected from the group consisting of diatomaceous earth, cationic and anionic colloidal particle suspensions.

6. The method according to Claim 4, wherein the first polysulfone membrane has a molecular cutoff of 30,000 daltons.

7. The method according to Claim 4, wherein the second polysulfone membrane has a molecular cutoff of 8,000 daltons.

8. The method according to Claim 1, wherein said cation exchange chromatographic step is accomplished with Sepharose.

9. The method according to Claim 1, wherein said cation exchange step involves elution from said cation exchange column with a step gradient of a salt selected from the group consisting of ammonium chloride, potassium chloride, and sodium chloride.

10. The method according to Claim 1, wherein said cation exchange chromatographic step includes elution with a buffer containing 1 to 6 molar urea.

11. The method according to Claim 1, wherein said reversed phase column is a resin having 4, 8, or 18 carbon atom chains.

12. The method according to Claim 1, wherein said reversed phase column is eluted with a step gradient of isopropanol and trifluoroacetic acid.

13. The method according to Claim 12, wherein said trifluoroacetic acid has a concentration ranging from 0.15 to 0.25%.

14. The method according to Claim 1, wherein said horseshoe crab is Limulus polyphemus.

15. A product produced by the method of Claim 1, wherein said product has endotoxin binding capability, and is a protein having an initial amino acid sequence selected from the group consisting of

Ser-Asn-Ile-Trp-Thr-,

Asp-Asn-,

Ser-Gly-, and

Ser-Asn-.

16. A method for ameliorating the biological effects of endotoxin in vivo, which comprises administering to a mammal in need of such treatment an effective amount of an endotoxin-binding protein of the horseshoe crab.

17. The method of Claim 16, wherein said endotoxin-binding protein has an initial amino acid sequence selected from the group consisting of

Ser-Asn-Ile-Trp-Thr-,

Asp-Asn-,

Ser-Gly-, and

Ser-Asn.

18. A method for ameliorating the biological effects of endotoxin in vivo, which comprises administering to a mammal in need of such treatment an effective amount of an endotoxin-binding protein preparation obtained from the horseshoe crab according to the following process:

(a) subjecting amebocytes obtained from a horseshoe crab to hypotonic shock to lyse said amebocytes, and obtain cell debris from said lysed amebocytes;

(b) extracting said cell debris with a solution containing a denaturant selected from the group consisting of urea and guanidine hydrochloride, to produce an extract;

(c) passing said extract through a first ultrafiltration membrane having a molecular cutoff of from 20,000 to 50,000 daltons, to obtain a filtrate;

(d) concentrating said filtrate by passing it through a second ultrafiltration membrane having a molecular cutoff of from 5,000 to 10,000, to produce a retentate;

(e) subjecting said retentate to cation exchange chromatography at a pH of from about 5 to 6, using an elution buffer which comprises urea, and eluting a solution containing a peak of endotoxin binding activity; and

(f) applying said solution containing said peak of endotoxin binding activity to a reverse phase column, and adding a buffer to said reverse phase column, to obtain a solution containing purified endotoxin binding protein.

19. The method according to Claim 18, wherein said hypotonic shock is accomplished by treating said amebocytes with endotoxin-free distilled water at about 0°C to 10°C.

20. The method according to Claim 18, wherein said extraction of said cell debris is accomplished with 6 molar urea or guanidine hydrochloride.

21. The method according to Claim 18, wherein said ultrafiltration membranes are each composed of polysulfone.

22. The method according to Claim 18, wherein before passing said extract through said membrane, the extract is crudely filtered with a filter aid selected from the group consisting of diatomaceous earth, cationic and anionic colloidal particle suspensions.

23. The method according to Claim 21, wherein the first polysulfone membrane has a molecular cutoff of 30,000 daltons.

24. The method according to Claim 21, wherein the second polysulfone membrane has a molecular cutoff of 8,000 daltons.

25. The method according to Claim 18, wherein said cation exchange chromatographic step is accomplished with Sepharose.

26. The method according to Claim 18, wherein said cation exchange step involves elution from said cation exchange column with a step gradient of salt selected from the group consisting of ammonium chloride, potassium chloride, and sodium chloride.

27. The method according to Claim 18, wherein said cation exchange chromatographic step includes elution with a buffer containing 1 to 6 molar urea.

28. The method according to Claim 18, wherein said reverse phase column is a resin having 4, 8, or 18 carbon atom chains.

29. The method according to Claim 18, wherein said reverse phase column is eluted with a step gradient of isopropanol and trifluoroacetic acid.

30. The method according to Claim 29, wherein said trifluoroacetic acid has a concentration ranging from 0.15 to 0.25%.

31. The method according to Claim 18, wherein said horseshoe crab is Limulus polyphemus.

32. The method according to Claim 16 or 18, wherein said endotoxin binding protein is administered intravenously.

33. The method according to Claim 16 or 18, wherein said mammal is a human.

34. The method according to Claim 16 or 18, wherein the dose of endotoxin binding protein is from about 0.1 to 100 mg of endotoxin binding protein per kg of body weight per day per patient.

35. A pharmaceutical composition effective for ameliorating the biological effects of endotoxin in vivo when administered to a mammal, comprising a purified endotoxin-binding protein of the horseshoe crab and a pharmaceutically acceptable carrier.

36. The pharmaceutical composition of Claim 35, wherein said endotoxin-binding protein has an initial amino acid sequence selected from the group consisting of

Ser-Asn-Ile-Trp-Thr-

Asp-Asn-,

Ser-Gly-, and

Ser-Asn.

37. A pharmaceutical composition effective for ameliorating the biological effects of endotoxin <u>in vivo</u> when administered to a mammal, comprising a purified endotoxin-binding protein preparation and a pharmaceutically acceptable carrier, wherein said protein preparation is obtained according to the following process:

(a) subjecting amebocytes obtained from a horseshoe crab to hypotonic shock to lyse said amebocytes, and obtain cell debris from said lysed amebocytes;

(b) extracting said cell debris with a solution containing a denaturant selected from the group consisting of urea and guanidine hydrochloride, to produce an extract;

(c) passing said extract through a first ultrafiltration membrane having a molecular cutoff of from 20,000 to 50,000 daltons, to obtain a filtrate;

(d) concentrating said filtrate by passing it through a second ultrafiltration membrane having a molecular cutoff of from 5,000 to 10,000, to produce a retentate;

(e) subjecting said retentate to cation exchange chromatography at a pH of from about 5 to 6, using an elution buffer which comprises urea, and eluting a solution containing a peak of endotoxin binding activity; and

(f) applying said solution containing said peak of endotoxin binding activity to a reverse phase column, and adding a buffer to said reverse phase column, to obtain a solution containing purified endotoxin binding protein.

38. The pharmaceutical composition according to Claim 37, wherein said hypotonic shock is accomplished by treating said amebocytes with endotoxin-free distilled water at about 0°C to 10°C.

39. The pharmaceutical composition according to Claim 37, wherein said extraction of said cell debris is accomplished with 6 molar urea or guanidine hydrochloride.

40. The pharmaceutical composition according to Claim 37, wherein said ultrafiltration membranes are each composed of polysulfone.

41. The pharmaceutical composition according to Claim 37, wherein before passing said extract through said membrane, the extract is crudely filtered with a filter aid selected from the group consisting of diatomaceous earth, cationic and anionic colloidal particle suspensions.

42. The pharmaceutical composition according to Claim 40, wherein the first polysulfone membrane has a molecular cutoff of 30,000 daltons.

43. The pharmaceutical composition according to Claim 40, wherein the second polysulfone membrane has a molecular cutoff of 8,000 daltons.

44. The pharmaceutical composition according to Claim 37, wherein said cation exchange chromatographic step is accomplished with Sepharose.

45. The pharmaceutical composition according to Claim 37, wherein said cation exchange step involves elution from said cation exchange column with a step gradient of salt selected from the group consisting of ammonium chloride, potassium chloride, and sodium chloride.

46. The pharmaceutical composition according to Claim 37, wherein said cation exchange chromatographic step includes elution with a buffer containing 1 to 6 molar urea.

47. The pharmaceutical composition according to Claim 37, wherein said reverse phase column is a resin having 4, 8, or 18 carbon atom chains.

48. The pharmaceutical composition according to Claim 37, wherein said reverse phase column is eluted with a step gradient of isopropanol and trifluoroacetic acid.

49. The pharmaceutical composition according to Claim 48, wherein said trifluoroacetic acid has a concentration ranging from 0.15 to 0.25%.

50. The pharmaceutical composition according to Claim 37, wherein said horseshoe crab is Limulus polyphemus.

51. A method for ameliorating the biological effects of endotoxin in vivo, which comprises administering to a

mammal in need thereof an effective amount of an endotoxin binding protein having amino acid sequence SEQ. ID. NO. I.

52. The method according to Claim 51, wherein said endotoxin binding protein is administered intravenously.

53. The method according to Claim 51, wherein said mammal is a human.

54. The method according to Claim 53, wherein the dose of endotoxin binding protein is from about 0.1 to 100 mg of endotoxin binding protein per kg of body weight per day per patient.

55. The method according to Claim 51, wherein said endotoxin binding protein is administered prophylactically to said mammal before the mammal is exposed to endotoxin.

56. The method according to Claim 51, wherein said endotoxin binding protein is administered to said mammal after the mammal is exposed to endotoxin.

57. A pharmaceutical composition for administration to a mammal to ameliorate the biological effects of endotoxin in vivo, which comprises an endotoxin binding protein having amino acid sequence SEQ. ID. NO. I in combination with a pharmaceutically acceptable carrier material.

58. A method for ameliorating the biological effects of endotoxin in vivo which comprises administering to a mammal in need thereof an effective amount of an endotoxin binding protein having an amino acid sequence corresponding at least to amino acid position 30 to 55 of SEQ. ID. NO. III and up to complete SEQ. ID. NO. III.

59. The method according to Claim 58, wherein said endotoxin binding protein is administered intravenously.

60. The method according to Claim 58, wherein said mammal is a human.

61. The method according to Claim 59, wherein the dose of endotoxin binding protein is from about 0.1 to 100 mg of endotoxin binding protein per kg of body weight day per patient.

62. The method according to Claim 58, wherein said endotoxin binding protein is administered prophylactically to said mammal before the mammal is exposed to endotoxin.

63. The method according to Claim 58, wherein said endotoxin binding protein is administered to said mammal after the mammal is exposed to endotoxin.

64. A pharmaceutical composition for administration to a mammal to ameliorate the biological effects of endotoxin in vivo, which comprises an endotoxin binding protein having an amino acid sequence corresponding at least to amino acid positions 30 to 55 of SEQ. ID. NO. III and up to complete amino SEQ. ID. NO. III, in combination with a pharmaceutically acceptable carrier material.

65. An endotoxin binding protein having amino acid sequence SEQ. ID. NO. I free of the contaminating components naturally associated with the horseshoe crab.

66. An endotoxin binding protein having an amino acid sequence corresponding at least to amino acid positions 30 to 55 of SEQ. ID. NO. III and up to complete amino SEQ. ID.

NO. III free of the contaminating components naturally associated with the horseshoe crab.

67. A DNA molecule coding for an endotoxin binding protein having amino acid sequence SEQ. ID. NO. I, wherein said DNA molecule is free of the contaminating components naturally associated with the horseshoe crab.

68. A DNA molecule coding for an endotoxin binding protein having an amino acid sequence corresponding at least to amino acid positions 30 to 55 of SEQ. ID. NO. III and up to complete amino SEQ. ID. NO. III, wherein said DNA molecule is free of the contaminating components naturally associated with the horseshoe crab.

69. The endotoxin binding protein of Claim 15, 65 or 66 immobilized on a solid phase support.

70. The immobilized endotoxin binding protein of Claim 69, wherein said solid phase support is a chromatographic resin or a membrane.

71. The immobilized endotoxin binding protein of Claim 69 which is a biosensor device.

72. The biosensor device of Claim 71, wherein said solid phase support is quartz or silicon.

73. A method for assaying endotoxin concentration, which comprises contacting serial aqueous dilutions of a material suspected of containing endotoxin with a known quantity of the endotoxin binding protein of claim 15, 65 or 66,

observing the fluorescence emission of at least one wavelength of the endotoxin binding protein before and after contact with the aqueous dilutions of said material suspected of containing endotoxin,

correlating the levels of fluorescence emission with at least one known emission level to thereby determine the quantity of endotoxin present in said material suspected of containing endotoxin.

74. The method of Claim 73, wherein said material is a body fluid.

75. The method of Claim 73, wherein said fluorescence emission of the endotoxin binding protein is measured at from 340 to 360 nanometers.

76. The method of Claim 73, wherein said fluorescence emission is produced by excitation at from about 275 to 295 nanometers.

77. A method for reducing endotoxin contamination of a material suspected of containing endotoxin, comprising contacting said material with the endotoxin binding molecule of claim 15, 65 or 66 to form a complex between endotoxin and the endotoxin binding molecule, and separating said complex from said sample.

78. The method of Claim 77, wherein said endotoxin binding protein is immobilized on a chromatographic resin or on a membrane.

79. A method for the extracoporeal removal of endotoxin from blood, comprising contacting blood with the immobilized endotoxin binding protein of Claim 69.

80. A method for assaying for endotoxin concentration in a material suspected of containing endotoxin, which comprises contacting said material with a biosensor device comprising the endotoxin binding protein of Claim 15, 65 or 66 immobilized on a solid phase support, detecting a change in capacitance, resistance, or acoustic wave of said solid phase support, and correlating the change with the changes observed with standard solutions of endotoxin binding protein.

81. The method of Claim 80, wherein said solid phase support is quartz or silicon.